AMENDMENTS TO CLAIMS

Applicants respectfully request that Claims 1-66 be cancelled, and that new Claims 70-84 be added.

Claim 1 (cancelled): A method of treating a mammal for a pre-cancerous or cancerous disease, wherein said disease is characterized by overexpression of MN/CA IX protein, comprising administering to said mammal a therapeutically effective amount of a composition comprising a compound, wherein said compound is selected from the group consisting of organic and inorganic molecules, and wherein said compound is determined to be a potent inhibitor of MN/CA IX enzymatic activity in a screening assay comprising:

a) preparing serial dilutions of said compound and serial dilutions of MN/CA IX protein or a fragment of the MN/CA IX protein that comprises the carbonic anhydrase domain;

b) preincubating a dilution of said compound with a dilution of said MN/CA IX protein or said MN/CA IX protein fragment for ten minutes at 20°C;

c) combining said preincubated mixture of said diluted compound and said diluted MN/CA IX protein or protein fragment with a substrate, consisting essentially of a saturated CO_2 solution, phenol red to 0.2mM, Na_2SO_4 to 0.1M, and Hepes buffer (pH 7.5) to 10mM, in a reaction vessel for a period of 10 to 100 seconds at 20°C;

d) concurrently measuring the optical density, at the absorbance maximum of 557 nm, of the contents of said reaction vessel, using a stopped flow spectrophotometer; and

e) determining the inhibition constant K_I of said compound;

wherein if said inhibition constant K_I is determined to be less than about 50 nanomolar, said compound is determined be a potent inhibitor of MN/CA IX enzymatic activity; and wherein said compound is not selected from the group consisting of acetazolamide, ethoxzolamide, methazolamide and cyanate.

Claim 2 (cancelled): The method of claim 1 wherein said mammal is a human.

Claim 3 (cancelled): The method of claim 1 wherein said inhibition constant K_I is determined to be less than about 35 nanomolar.

Claim 4 (cancelled): The method of claim 1 wherein said inhibition constant K_I is determined to be less than about 10 nanomolar.

Claim 5 (cancelled): The method of claim 1 wherein said compound is an organic compound.

Claim 6 (cancelled): The method of claim 1 wherein said compound is an inorganic compound.

Claim 7 (cancelled): The method of claim 5 wherein said organic compound is an aromatic compound.

Claim 8 (cancelled): The method of claim 5 wherein said organic compound is an aromatic sulfonamide or a heterocyclic sulfonamide.

Claim 9 (cancelled): The method of claim 8, wherein said aromatic sulfonamide is a substituted aromatic sulfonamide, wherein said aromatic sulfonamide comprises an aromatic ring structure bearing a sulfonamide moiety bonded to said ring structure and optionally bearing one or more substituents independently selected from the group consisting of halogeno, nitro, and an alkylamino group, wherein the alkyl radical of said alkylamino group comprises 1 to 4 carbon atoms.

Claim 10 (cancelled): The method of claim 8 wherein said compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of a carbonic anhydrase selected from the group consisting of CA I, CA II and CA IV.

Claim 11 (cancelled): The method of claim 8 wherein said compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of at

least two carbonic anhydrases selected from the group consisting of CA I, CA II and CA IV.

Claim 12 (cancelled): The method of claim 8 wherein said compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of each of the carbonic anhydrases in the group consisting of CA I, CA II and CA IV.

Claim 13 (cancelled): The method of claim 8 wherein said compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of CA II.

Claim 14 (cancelled): The method of claim 13 wherein the inhibition by said compound of the enzymatic activity of CA II is tested by the method comprising the following steps:

a) preparing serial dilutions of said compound and serial dilutions of CA II;

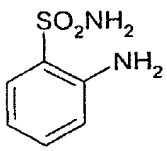
b) preincubating a dilution of said compound with a dilution of CA II for ten minutes at 20°C;

c) adding said preincubated mixture of said compound and said CA II to a substrate solution, comprising 4-nitrophenylacetate in anhydrous acetonitrile (pH 7.40), in a reaction vessel for a period of 1 to 3 minutes at 25°C;

d) concurrently measuring the optical density, at the absorbance maximum of 400 nm, of the contents of said reaction vessel, using a spectrophotometer; and

e) determining the inhibition constant K_I of said compound.

Claim 15 (cancelled): The method of claim 8 wherein said aromatic sulfonamide or heterocyclic sulfonamide is selected from the group consisting of:



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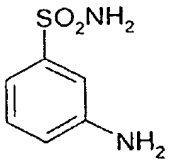

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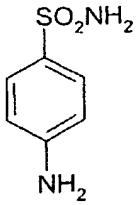

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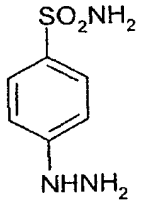

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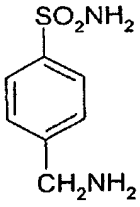

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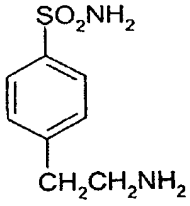

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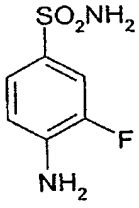

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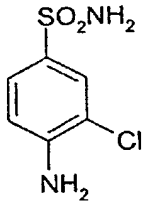

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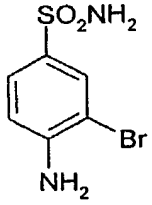

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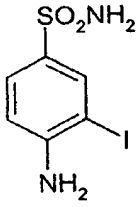

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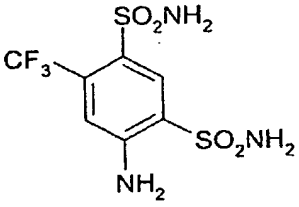

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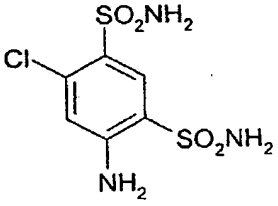

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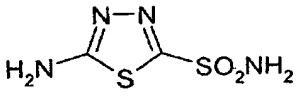

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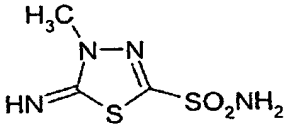

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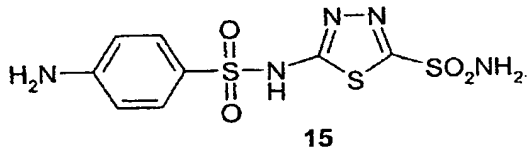

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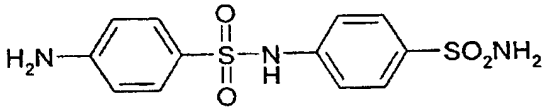

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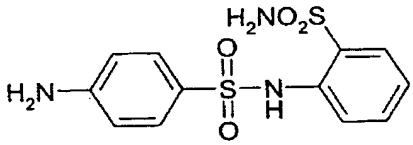

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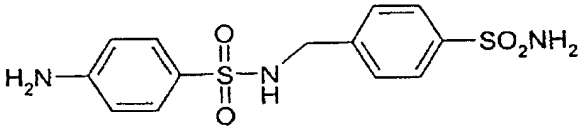

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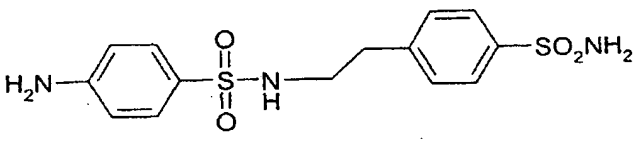

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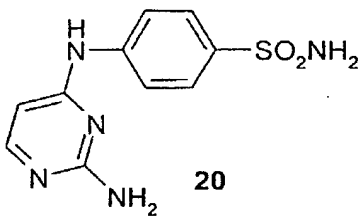

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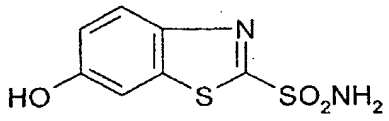

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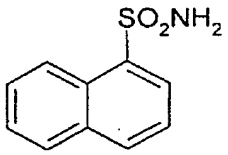

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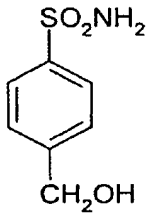

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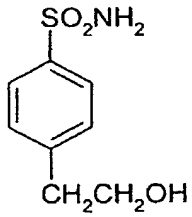

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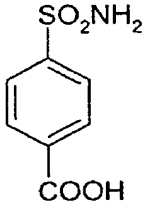

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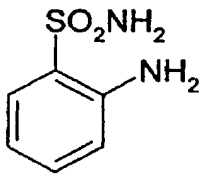
Claim 16 (cancelled): The method of claim 8, wherein said compound is an aromatic sulfonamide selected from the group consisting of:



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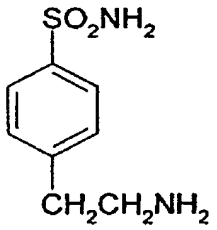

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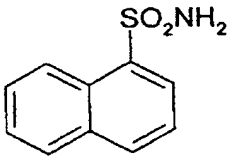

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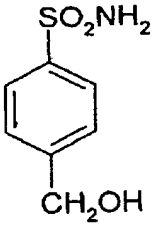

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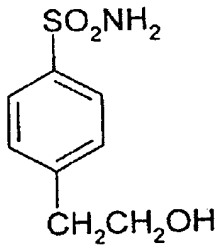

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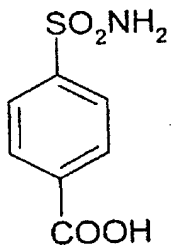

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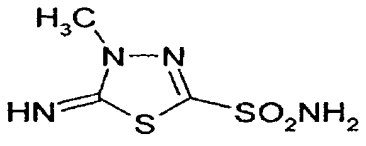
Claim 17 (cancelled): The method of claim 8, wherein a halogen atom is bonded to at least one carbon atom in the aromatic ring of said aromatic sulfonamide.

Claim 18 (cancelled): The method of claim 8, wherein said compound is a heterocyclic sulfonamide.

Claim 19 (cancelled): The method of claim 18, wherein said heterocyclic compound is a substituted heterocyclic sulfonamide, wherein said substituted heterocyclic sulfonamide comprises a heterocyclic ring structure bearing an sulfonamide moiety bonded to said ring structure and optionally bearing one or more substituents independently selected from a group consisting of halogeno, nitro, and an alkylamino group, wherein the alkyl radical of said alkylamino group comprises 1 to 4 carbon atoms.

Claim 20 (cancelled): The method of claim 18, wherein said heterocyclic sulfonamide is halogenated.

Claim 21 (cancelled): The method of claim 8, wherein said compound is a heterocyclic sulfonamide selected from the group consisting of:



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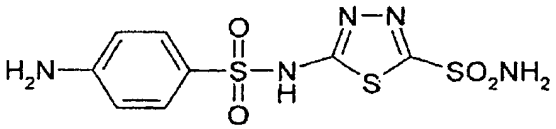

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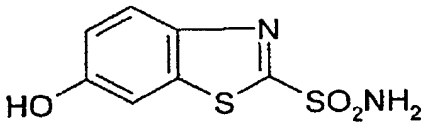

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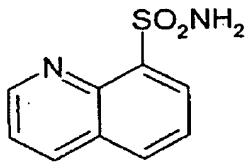

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Claim 22 (cancelled): A method of treating a mammal for a pre-cancerous or cancerous disease, wherein said disease is characterized by overexpression of MN/CA IX protein, comprising administering to said mammal a therapeutically effective amount of a composition comprising a membrane-impermeant compound, wherein said membrane-impermeant compound is selected from the group consisting of organic and inorganic molecules, and wherein said membrane-impermeant compound is determined to be a potent inhibitor of MN/CA IX enzymatic activity in a screening assay comprising:

a) preparing serial dilutions of said membrane-impermeant compound and serial dilutions of MN/CA IX protein or a fragment of the MN/CA IX protein that comprises the carbonic anhydrase domain;

b) preincubating a dilution of said membrane-impermeant compound with a dilution of said MN/CA IX protein or said MN/CA IX protein fragment for ten minutes at 20°C;

c) combining said preincubated mixture of said diluted compound and said diluted MN/CA IX protein or protein fragment with a substrate, consisting essentially of a saturated CO_2 solution, phenol red to 0.2mM, Na_2SO_4 to 0.1M, and Hepes buffer (pH 7.5) to 10mM, in a reaction vessel for a period of 10 to 100 seconds at 20°C;

d) concurrently measuring the optical density, at the absorbance maximum of 557 nm, of the contents of said reaction vessel, using a stopped flow spectrophotometer; and

e) determining the inhibition constant K_I of said membrane-impermeant compound,

wherein if said inhibition constant K_I is determined to be less than about 50 nanomolar, said membrane-impermeant compound is determined be a potent inhibitor of MN/CA IX enzymatic activity.

Claim 23 (cancelled): The method of claim 22 wherein said mammal is a human.

Claim 24 (cancelled): The method of claim 22 wherein said inhibition constant K_I is determined to be less than about 35 nanomolar.

Claim 25 (cancelled): The method of claim 22 wherein said inhibition constant K_I is determined to be less than about 10 nanomolar.

Claim 26 (cancelled): The method of claim 22 wherein said membrane-impermeant compound is an organic compound.

Claim 27 (cancelled): The method of claim 22 wherein said membrane-impermeant compound is an inorganic compound.

Claim 28 (cancelled): The method of claim 26 wherein said membrane-impermeant organic compound is a pyridinium derivative of an aromatic sulfonamide or a pyridinium derivative of a heterocyclic sulfonamide.

Claim 29 (cancelled): The method of claim 28 wherein said membrane-impermeant compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of a carbonic anhydrase selected from the group consisting of CA I, CA II and CA IV.

Claim 30 (cancelled): The method of claim 28 wherein said membrane-impermeant compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of at least two carbonic anhydrases selected from the group consisting of CA I, CA II and CA IV.

Claim 31 (cancelled): The method of claim 28 wherein said membrane-impermeant compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of each of the carbonic anhydrases in the group consisting of CA I, CA II and CA IV.

Claim 32 (cancelled): The method of claim 28 wherein said membrane-impermeant compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of CA IV.

Claim 33 (cancelled): The method of claim 32 wherein the inhibition by said membrane-impermeant compound of the enzymatic activity of CA IV is tested by the method comprising the following steps:

a) preparing serial dilutions of said membrane-impermeant compound and serial dilutions of CA IV;

b) preincubating a dilution of said membrane-impermeant compound with a dilution of CA IV for ten minutes at 20°C;

c) adding said preincubated mixture of said compound and said CA IV to a substrate solution, comprising 4-nitrophenylacetate in anhydrous acetonitrile (pH 7.40), in a reaction vessel for a period of 1 to 3 minutes at 25°C;

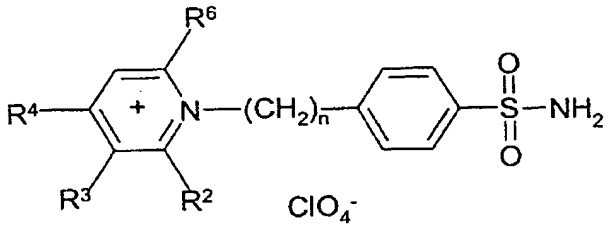
d) concurrently measuring the optical density, at the absorbance maximum of 400 nm, of the contents of said reaction vessel using a spectrophotometer; and

e) determining the inhibition constant K_I of said membrane-impermeant compound.

Claim 34 (cancelled): The method of claim 28, wherein said membrane-impermeant compound is a pyridinium derivative of an aromatic sulfonamide.

Claim 35 (cancelled): The method of claim 34, wherein said membrane-impermeant compound is a pyridinium derivative of an aromatic sulfonamide that is selected from the group consisting of sulfanilamide, homosulfanilamide and 4-aminoethyl-benzenesulfonamide.

Claim 36 (cancelled): The method of claim 34, wherein said pyridinium derivative of an aromatic sulfonamide has the general formula of:



wherein

n is 0, 1, or 2;

R2, R3, R4 and R6 are each independently selected from the group consisting of

hydrogen, alkyl moieties comprising from 1 to 12 carbon atoms, and aryl moieties.

Claim 37 (cancelled): The method of claim 36 wherein

R2 is selected from the group consisting of methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *tert*-butyl and phenyl;

R3 is selected from the group consisting of hydrogen and methyl;

R4 is selected from the group consisting of hydrogen, methyl and phenyl;

and

R6 is selected from the group consisting of methyl, ethyl, *n*-propyl, *iso*-propyl, and phenyl.

Claim 38 (cancelled): The method of claim 37, wherein

R3 is hydrogen;

R4 and R6 are phenyl;

when n is 0, R2 is selected from the group consisting of methyl, ethyl, n-propyl, iso-propyl, n-butyl, and phenyl; and

when n is 1 or 2, R2 is selected from the group consisting of methyl, ethyl, n-propyl, iso-propyl, n-butyl, tert-butyl, and phenyl.

Claim 39 (cancelled): The method of claim 37, wherein

R3 is hydrogen;

R4 is phenyl; and

when n is 0, R2 and R6 are the same and are selected from the group consisting of methyl, ethyl, n-propyl, and iso-propyl; and

when n is 1 or 2, R2 and R6 are the same and are selected from the group consisting of methyl, ethyl, n-propyl, iso-propyl and phenyl.

Claim 40 (cancelled): The method of claim 37, wherein R2, R3, R4 and R6 are methyl.

Claim 41 (cancelled): The method of claim 37, wherein

when n is 0, 1 or 2, R2, R4 and R6 are methyl, and R3 is hydrogen; or

when n is 1 or 2, R2 is iso-propyl, R3 is hydrogen, R4 is methyl, and R6 is methyl or iso-propyl; or

when n is 2, R2 and R6 are phenyl, and R3 and R4 are hydrogen.

Claim 42 (cancelled): The method of claim 37, wherein

when n is 2, R2 and R6 are methyl, R3 is hydrogen, and R4 is phenyl; or

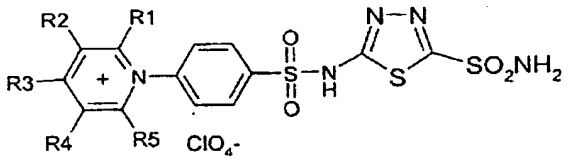
when n is 2, R2 and R6 are ethyl, R3 is hydrogen, and R4 is phenyl; or

when n is 2, R2, R3, R4 and R6 are methyl.

Claim 43 (cancelled): The method of claim 28, wherein said membrane-impermeant compound is a pyridinium derivative of a heterocyclic sulfonamide.

Claim 44 (cancelled): The method of claim 43, wherein said membrane-impermeant compound is a pyridinium derivative of aminobenzolamide.

Claim 45 (cancelled): The method of claim 43, wherein said pyridinium derivative of a heterocyclic sulfonamide has the general formula of:



wherein R1, R2, R3, R4 and R5 are each independently selected from the group consisting of hydrogen, alkyl moieties comprising from 1 to 12 carbon atoms, and aryl moieties.

Claim 46 (cancelled): The method of claim 45, wherein

R1 is selected from the group consisting of methyl, ethyl, iso-propyl, n-propyl, n-butyl, *tert*-butyl and phenyl;

R2 is selected from the group consisting of hydrogen and methyl;

R3 is selected from the group consisting of hydrogen, methyl, *n*-nonyl, and phenyl;

R4 is hydrogen; and

R5 is selected from the group consisting of methyl, ethyl, iso-propyl, n-propyl, n-butyl, *tert*-butyl, *n*-nonyl,and phenyl.

Claim 47 (cancelled): The method of claim 46, wherein

R2 and R4 are hydrogen;

R3 is methyl; and

R1 and R5 are the same and selected from the group consisting of methyl, *iso*-propyl, and *tert*-butyl.

Claim 48 (cancelled): The method of claim 46, wherein

R2 and R4 are hydrogen;

R3 is phenyl; and

R1 and R5 are the same and selected from the group consisting of methyl, ethyl, iso-propyl, n-propyl, n-butyl, and phenyl.

Claim 49 (cancelled): The method of claim 46, wherein

R1 is selected from the group consisting of methyl, ethyl, iso-propyl, n-propyl, and n-butyl;

R2 and R4 are hydrogen; and

R3 and R5 are phenyl.

Claim 50 (cancelled): The method of claim 46, wherein

R2 and R4 are hydrogen, R3 is hydrogen or methyl, and R1 and R5 are phenyl; or

R1, R2, and R5 are methyl, R3 is phenyl, and R4 is hydrogen; or

R1 is methyl, R2 and R4 are hydrogen, and R3 and R5 are n-nonyl.

Claim 51 (cancelled): The method of claim 46, wherein

R1 is methyl or iso-propyl, R3 and R5 are methyl, and R2 and R4 are hydrogen; or

R1 and R5 are the same and are methyl or ethyl, R2 and R4 are hydrogen, and R3 is phenyl; or

R1, R2, R3 and R5 are methyl and R4 is hydrogen.

Claim 52 (cancelled): A method of inhibiting tumor growth in a patient having a tumor, the cells of which tumor are characterized by overexpression of MN/CA

IX protein, comprising administering to said patient a therapeutically effective amount of a composition comprising a compound, wherein said compound is selected from the group consisting of organic and inorganic molecules, and wherein said compound is determined to be a potent inhibitor of MN/CA IX enzymatic activity in a screening assay comprising:

a) preparing serial dilutions of said compound and serial dilutions of MN/CA IX protein or a fragment of the MN/CA IX protein that comprises the carbonic anhydrase domain;

b) preincubating a dilution of said compound with a dilution of said MN/CA IX protein or protein fragment for ten minutes at 20°C;

c) combining said preincubated mixture of said diluted compound and said diluted MN/CA IX protein or protein fragment with a substrate, consisting essentially of a saturated CO_2 solution, phenol red to 0.2mM, Na_2SO_4 to 0.1M, and Hepes buffer (pH 7.5) to 10mM, in a reaction vessel for a period of 10 to 100 seconds at 20°C;

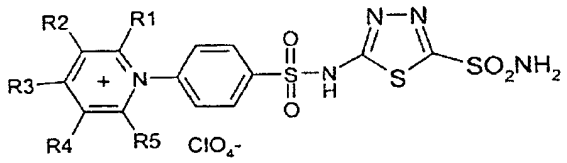
d) concurrently measuring the optical density, at the absorbance maximum of 557 nm, of the contents of said reaction vessel, using a stopped flow spectrophotometer; and

e) determining the inhibition constant K_I of said compound;

wherein if said inhibition constant K_I is determined to be less than about 50 nanomolar, said compound is determined be a potent inhibitor of MN/CA IX enzymatic activity; and wherein said compound is not selected from the group consisting of acetazolamide, ethoxzolamide, methazolamide and cyanate.

Claim 53 (cancelled): The method of claim 52 wherein said patient is a human.

Claim 54 (cancelled): A pyridinium derivative of a heterocyclic sulfonamide with the general formula of:



wherein

R1 is selected from the group consisting of methyl, ethyl, iso-propyl, *n*-propyl, *n*-butyl, *tert*-butyl and phenyl;

R2 is selected from the group consisting of hydrogen and methyl;

R3 is selected from the group consisting of hydrogen, methyl, *n*-nonyl and phenyl;

R4 is hydrogen; and

R5 is selected from the group consisting of methyl, ethyl, iso-propyl, n-propyl, n-butyl, *tert*-butyl, *n*-nonyl and phenyl, except that

R1 cannot be methyl when R2 and R4 are hydrogen and R3 and R5 are methyl; and

R1 cannot be methyl when R2 and R4 are hydrogen, R3 is phenyl and R5 is methyl; and

R1 cannot be phenyl when R2 and R4 are hydrogen and R3 and R5 are phenyl.

Claim 55 (cancelled): The pyridinium derivative of a heterocyclic sulfonamide of claim 54, wherein

R2 and R4 are hydrogen;

R3 is methyl; and

R1 and R5 are the same and selected from the group consisting of *iso*-propyl and *tert*-butyl.

Claim 56 (cancelled): The pyridinium derivative of a heterocyclic sulfonamide of claim 54, wherein

R2 and R4 are hydrogen;

R3 is phenyl; and

R1 and R5 are the same and selected from the group consisting of ethyl, iso-propyl, n-propyl, and n-butyl.

Claim 57 (cancelled): The pyridinium derivative of a heterocyclic sulfonamide of claim 54, wherein

R1 is selected from the group consisting of methyl, ethyl, iso-propyl, n-propyl, n-butyl, and *tert*-butyl;

R2 and R4 are hydrogen; and

R3 and R5 are phenyl.

Claim 58 (cancelled): The pyridinium derivative of a heterocyclic sulfonamide of claim 54, wherein

R1 is iso-propyl, R3 and R5 are methyl, and R2 and R4 are hydrogen; or

R2 and R4 are hydrogen, R3 is hydrogen or methyl, and R1 and R5 are phenyl; or

R1, R2, and R5 are methyl, R3 is phenyl, and R4 is hydrogen; or

R1, R2, R3 and R5 are methyl and R4 is hydrogen; or

R1 is methyl, R3 and R5 are *n*-nonyl, and R2 and R4 are hydrogen.

Claim 59 (cancelled): The method of claim 1 further comprising conjugating a radioisotope to said compound before administering said compound to said mammal.

Claim 60 (cancelled): The method of claim 1 further comprising administering to said mammal radiation and/or a therapeutically effective amount in a physiologically acceptable formulation of one or more of the following compounds selected from the group consisting of: conventional anticancer drugs, chemotherapeutic agents, different inhibitors of cancer-related pathways, bioreductive drugs, CA IX-specific antibodies and CA IX-specific antibody fragments that are biologically active.

Claim 61 (cancelled): The method of claim 60 wherein said CA IX-specific antibodies and/or CA IX-specific antibody fragments are humanized or fully human.

Claim 62 (cancelled): The method of claim 60 wherein said CA IX-specific antibodies and/or CA IX-specific antibody fragments are attached to a cytotoxic entity.

Claim 63 (cancelled): A method of treating a mammal for a precancerous or cancerous disease, wherein said disease is characterized by overexpression of MN/CA IX protein, comprising administering to said mammal a therapeutically effective amount in a physiologically acceptable formulation of a vector conjugated to a potent CA IX-specific inhibitor, wherein said vector expresses a wild-type gene that is absent from or mutated in a CA IX expressing cell, that is precancerous or cancerous, and wherein the wild type gene product has an anticancer effect in said cell; or

wherein said vector comprises a gene that expresses a cytotoxic protein.

Claim 64 (cancelled): The method according to claim 63 wherein said vector comprises a MN/CA IX promoter or a MN/CA IX promoter fragment, wherein said promoter or promoter fragment comprises one or more hypoxia response elements, and wherein said promoter or promoter fragment is operably linked to said wild-type gene or to said gene that expresses a cytotoxic protein.

Claim 65 (cancelled): The method of claim 63 wherein said potent CA IX-specific inhibitor is a compound determined to inhibit CA IX enzymatic activity in a screening assay comprising:

a) preparing serial dilutions of said compound and serial dilutions of MN/CA IX protein or a MN/CA IX protein fragment that comprises the carbonic anhydrase domain;

b) preincubating a dilution of said compound with a dilution of said MN/CA IX protein or said MN/CA IX protein fragment for ten minutes at 20°C;

c) combining said preincubated mixture of said diluted compound and